

Biosensing using straight long-range surface plasmon waveguides

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Abstract: Straight long-range surface plasmon waveguides are demonstrated as biosensors for the detection of cells, proteins and changes in the bulk refractive index of solutions. The sensors consist of 5 μm wide 22 nm thick Au stripes embedded in polymer (CYTOPTM) with microfluidic channels etched into the top cladding. Bulk sensing is demonstrated by sequentially injecting six solutions of different refractive indices in 2×10^{-3} RIU increments; such index steps were detected with a signal-to-noise ratio of ~ 1000 . Selective capture of cells is demonstrated using Au waveguides functionalized with antibodies against blood group A, and red blood cells of group A and O in buffer as positive and negative analyte. Bovine serum albumin in buffer was used to demonstrate protein sensing. A monolayer of bovine serum albumin physisorbed on a carboxyl-terminated self-assembled monolayer on Au was detected with a signal-to-noise ratio of ~ 300 . Overall, the biosensor demonstrated a good capability for detecting bulk changes in solution and for sensing analyte over a very wide range of mass (from cells to proteins). The biosensors are compact, inexpensive to fabricate, and may find use over a wide range of cost-sensitive sensing and detection applications.

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OCIS codes: (280.1415) Biological sensing and sensors; (240.6680) Surface plasmons; (130.3120) Integrated optics devices; (230.7390) Waveguides, planar; (250.5403) Plasmonics.

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1. Introduction

The field of optical biosensors is currently dominated by the methods of Surface Plasmon Resonance (SPR). Conventional SPR systems utilize a Kretschmann-Raether configuration, where a prism with a thin layer of Au is interrogated with a transverse magnetic (TM) polarized beam [1]. Real-time and label-free detection, and the small amount of required

ligand on the surface and analyte in solution, make SPR very appealing for drug discovery [2]. Real-time monitoring of reactions allows the extraction of biochemical interaction kinetics, while label-free sensing avoids unnecessary chemical manipulation of analyte or/and receptor molecules.

This paper presents a novel optical biosensor based on long-range surface plasmon-polariton (LRSP) waveguides. LRSPs are propagating plasmon waves that can be excited by TM-polarized light on thin symmetric metal stripes or slabs, and that can propagate over appreciable lengths [3]. The LRSP is a symmetric coupled mode formed by the coupling of single-interface SPPs through the thin metal film. The metal stripe provides optical confinement in the plane transverse to the direction of propagation, leading to the realization of a number of different integrated components such as S-bends, Y-junctions, couplers and Mach-Zehnder Interferometers (MZIs) [4,5]. LRSP excitation can be easily achieved by butt-coupling an optical single-mode fiber to the metal stripe, which can lead to compact structures and miniaturization of biosensors.

Although, LRSPs are less confined and less surface sensitive than single-interface SPPs, they propagate much farther so long-interaction length sensors providing greater adlayer sensitivity and a lower detection limits are possible [6]. Also, the sensing depth is greater ($\sim 1\ \mu\text{m}$ vs. $\sim 200\ \text{nm}$) so greater loading is possible, say via the use of a dextran hydrogel matrix to capture more proteins along this dimension [7]. Finally, LRSPs are also useful for sensing large biological entities such as cells which cause strong scattering of loosely bound LRSPs into radiative modes.

In a butt-coupling arrangement, the lowest insertion loss for an LRSP waveguide occurs when the refractive index around the metal stripe is homogeneous [8] (the insertion loss depends on the coupling efficiency and the attenuation of the mode). In order to match the refractive index of biologically compatible fluids, which have $n \approx 1.33$, low index claddings are needed. An advantage of using low index claddings is that fluidic channels become optically less invasive once filled with sensing solution. Cytop (Asahi) and Teflon (Dupont) are suitable and both have been used in sensing experiments involving LRSPs on metal slabs in prism-coupled geometries [9–14]. Cytop has also been used as a dielectric waveguide sensor [15], as have other dielectric materials such as silicon nitride [16–18] and silicon on insulator [19].

The sensors presented in this work consist of straight Au waveguides embedded in Cytop with an etched microfluidic channel providing access to the Au surfaces. These structures differ from previous ones involving LRSPs in that the latter are based on metal slabs which provide no lateral confinement. They also differ from dielectric waveguides in that the mode of operation is a surface plasmon and a metal stripe is used as the “core”. One rationale behind using Au waveguides instead of dielectric waveguides is the easy integration of surface chemistries for well-controlled functionalization. Generally Au is considered a preferable material for functionalization for a number of reasons: *i*) it is historically most studied, *ii*) organized packing of an alkanethiol self-assembled monolayer (SAM) can be easily achieved (organization and packing improves as the Au surface tends toward single-crystal 111), *iii*) SAMs formed on Au from thiols are stable for long periods of time, *iv*) SAMs form quickly through a simple incubation process: 80-90% of the SAM forms in a matter of minutes, and *v*) Au is a relatively inert material that does not oxidize below its melting temperature and does not react with most chemicals [20]. SAM formation on SiO_2 , for example, requires alkylsilanes, which are harder to use and process due to their moisture sensitivity. Other rationale for the use of Au waveguides is the provision of additional functionality, such as electrochemical desorption for topo-selective functionalization [21,22], and thermo-optic modulation [23,24] such that signal processing techniques could be used to improve the signal-to-noise ratio.

The purpose of this paper is to demonstrate the capabilities of straight LRSP waveguides for sensing large biological objects such as cells as well as small biochemical entities such as

proteins. We also investigate the effects of bulk refractive index changes on the performance of the waveguides. Selective capture of cells is important in a number of applications in medicine, for example, for rare circulating tumor cell (CTC) detection in blood [25]. Protein sensing is the most common strategy used for pathogen detection and drug discovery. Bulk sensing can be implemented for the precise determination of the refractive index of solutions, and its effects must be known in order to avoid them during biochemical sensing.

2. Materials and methods

2.1 Sensing platform

The sensors were fabricated as described in [26,27]. Cytop was spin-coated and cured on a 4" Si wafer to form the bottom cladding. Au features were defined lithographically by Au evaporation and lift-off. The Cytop upper cladding was then spin-coated and microfluidic channels were defined lithographically and etched down to the Au stripe surface. The wafer was then covered with resist for protection and diced into ~300 dies, each die containing 16 straight waveguides.

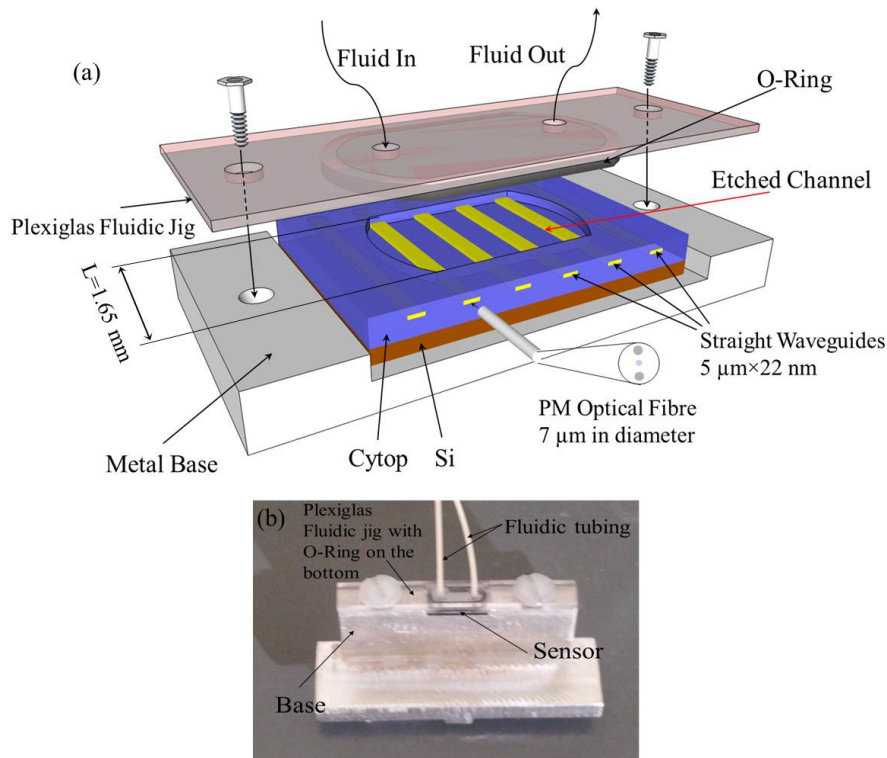


Fig. 1. Sensing device with integrated fluidics: a) schematic of the device placed on the metal base with a Plexiglas jig on top; the volume of the fluidic cell is 20 μL ; b) image of the device with fluidics fixed on the metal base.

A sensing die (6.5 mm wide by 3.8 mm long) is sketched in Fig. 1 and consists of LRSP waveguides each formed from 5 μm wide ~22 nm thick Au stripes embedded in Cytop with a large fluidic channel etched into the top cladding. The sensing length (etched part) of a waveguide is $L = 1.65$ mm. Other waveguide structures which are not shown were also present on the die interspersed between the straight waveguides.

A custom made fluidic jig consisting of a Plexiglas slide, with two holes for fluidic tubing, and an O-Ring (Apple Rubber Products Inc.) matched in dimensions to the etched

channel and attached on the bottom surface of the slide, provides a good seal and fluid exchange within the fluidic channel. A machined metal base (Al) is used to support the die and to integrate the sensor/fluidic assembly into the interrogation setup (Sec 2.2). The fluidic jig and the metal base are fixed together with screws leaving space at the front of the assembly to insert the optical fiber used to excite a waveguide sensor.

2.2 Interrogation setup

As a light source, a laser diode (NLK1356STG, $\lambda_0 = 1310$ nm, NTT Electronics) was used to provide an optical signal, carried by a polarization-maintaining (PM) optical fiber with a core diameter of 7 μm (PMJ-3AX-1300-7/125-1-1-1, OZ Optics). The diode output was controlled by a laser diode controller (LDC 3724B, ILX Lightwave). Two multi-axis positioning stages (Thorlabs Inc.) were used to align the fiber to the waveguide: a 6-axis stage to manipulate the fiber holder with the fiber and a 3-axis stage to manipulate the device under test (DUT) (*i.e.*, the sensor/fluidics assembly). A 25 $\times$ objective lens (Melles Griot) was permanently fixed to the table, defining the optical axis of the output portion of the setup and used to magnify and collimate the optical signal emerging from the DUT. The background light was removed by an aperture and the beam split into two portions, one sent to an infrared camera to visually monitor the alignment procedure and observe changes in output during an experiment, and the other to a power meter (81618A, Hewlett Packard). Labview was used to control the setup and perform data acquisition. The fluid was supplied by a syringe pump (PicoPlus, Harvard Apparatus) through pico tubing (550 μm outer diameter, 250 μm inner diameter, IDEX), connected to the fluidic holes of the plexiglas jig. The setup is sketched in Fig. 2.

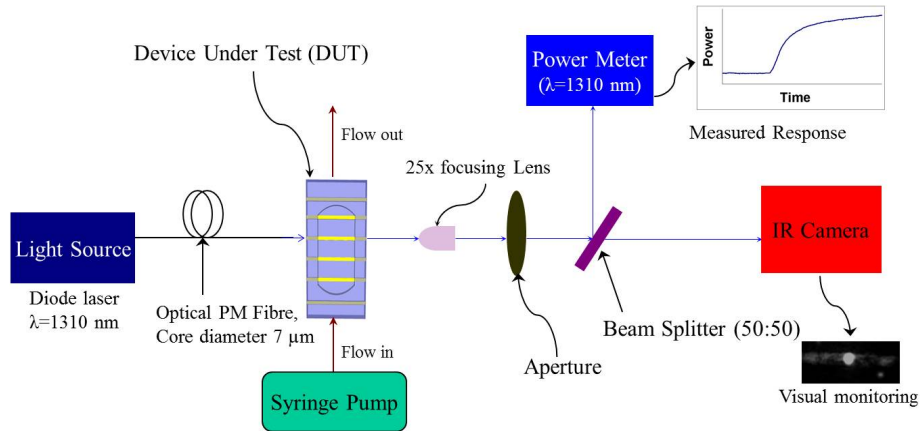


Fig. 2. Schematic representation of the interrogation setup with the sensing device.

2.3 Materials

2-Isopropanol semiconductor grade (IPA), 16-Mercaptohexadecanoic acid ($\text{HS}(\text{CH}_2)_{15}\text{COOH}$), triethylene glycol mono-11 mercaptoundecyl ether ($\text{HS}(\text{CH}_2)_{11}(\text{O C}_2\text{H}_4)_3\text{OH}$), *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC), *N*-Hydroxysuccinimide sodium salt (NHS), acetone HPLC grade $\geq 99.9\%$, Bovine Serum Albumin (BSA) and glycerol (electrophoresis grade), Phosphate buffer saline 0.01 M, pH 7.4 (PBS) were obtained from Sigma-Aldrich. PBS solution was prepared from the package by dissolving containing salts in 1 L of deionized water producing a buffer of the following constitution: 0.01 M phosphate buffer saline, 0.138 M NaCl and 0.0027 M KCl (according to the manufacturer). Packed human red blood cells (groups A and O) and antibodies against blood group A (Anti-A, Murine Monoclonal, Series 1, Immucor) were donated by Mount Sinai Hospital (Toronto). Distilled water was deionized using Millipore filtering membranes (Millipore, Milli-Q water system at 16 $\text{M}\Omega\cdot\text{cm}$).

2.4 Device cleaning and functionalization

The dicing photoresist was removed from an individual die by two sequential acetone baths (5 and 30 min), then by thoroughly washing in IPA. The cleanliness of the facets is of great importance to optical input and output coupling. Particulate matter, deposited during dicing, was removed by acetone and IPA ultrasonic baths (FS20H, Fisher Scientific) each of 1 min in duration. The sample was dried under N_2 . Further removal of possible organic matter on Au stripes was performed in a 1:1 solution of 50 mM KOH:H₂O₂ (30%) [28] for 2 min followed by an intense wash in distilled/deionized water (DDI H₂O), then in IPA, followed by drying with N_2 . Two types of alkanethiols were used in experiments to form SAMs: 16-mercaptohexadecanoic acid (16-MHA) for antibody functionalization or protein physisorption, and triethylene glycol mono-11 mercaptoundecyl ether (T-PEG) for avoiding non-specific adsorption. In both cases, the device was placed in a 2 mM IPA alkanethiol solution allowing the SAM formation to proceed for 12-18 hours.

For analysis of the BSA adsorption on different surfaces, a device after incubation in an alkanethiol solution was washed with plenty of IPA, dried under N_2 and assembled into the setup. Sensing of the refractive index of the bulk was performed with Au stripes functionalized with 16-MHA. For selective capture of RBCs, prior to the device integration into the setup, Anti-A antibodies were attached to the 16-MHA SAM using NHS/EDC amine coupling chemistry [29]. The carboxyl group of 16-MHA was activated by placing the device in a DDI H₂O solution of 0.1M EDC/NHS (1:1) for 15 minutes. After rinsing with DDI H₂O the chip was placed into the solution of Anti-A (in PBS) for ~2 hrs. Upon completion of the conjugation reaction, the device was washed with plenty of PBS (pH 7.4, 0.01M phosphate) and assembled into the setup.

2.5 Analyte preparation

To investigate the effects of the refractive index of bulk solutions, six DDI H₂O/Glycerol (DDI/Gly) mixtures were prepared with index increments of $\pm 2 \times 10^{-3}$ RIU, about $n = 1.3348$, which is the refractive index of Cytop; the index of the solutions were 1.3282, 1.3303, 1.3325, 1.3346, 1.3367 and 1.3409 as measured at $\lambda_0 = 1312$ nm using a prism-coupler based instrument (Model 2010, Metricon, Prism 200-P1). For all biosensing experiments, a mixture of biological buffer (PBS, pH = 7.4, 0.01 M phosphate) doped with 7.235% w/w glycerol (PBS/Gly) having an index of $n = 1.3303$ was used; the reason for using a sensing solution with an index slightly lower than that of Cytop will become apparent in Section 3.1.

Packed RBCs (group A and O), stored at 4 °C, were diluted with filtered PBS/Gly buffer and washed in a micro-centrifuge (Micromax, Thermo Electric Corp.) 3 times for 2 minutes (400 g) discarding the supernatant each time. Cells were counted using Hemacytometer (1483, Hausser Scientific).

BSA solutions were prepared by mixing lyophilized BSA with PBS/Gly buffer to a concentration of 100 μ g/ml.

2.6 Sensing assay using the platform

Prior to device installation into the setup, the TM-polarization alignment of the fiber was ensured and the stability of the setup was verified over a period of 10 minutes with the beam emerging from the fiber and propagating directly through the output portion of the setup (*i.e.*, no DUT). The device was then assembled with fluidics, PBS/Gly buffer ($n = 1.3303$) was introduced into the channel and tubing, and the device/fluidic assembly was inserted into the setup. The fiber was butt-coupled to a waveguide of good quality based on the symmetry of the emerging mode (as observed with the IR camera) and the measured power output. In order to avoid Fabry-Perot interferences between the fiber and the input facet, a glass index-matching oil was used between the two surfaces (Series AA, $n = 1.456$, Cargille). Every time

before introducing a solution with analyte, a baseline with flowing PBS/Gly buffer was established for 10 minutes to ensure stability and extract the noise level. Flow-rates and input powers varied for different experiments, but the wavelength was kept constant at $\lambda_0 = 1310$ nm. All solutions were filtered through Millex-GP filters (PES membrane 0.22 μm).

3. Results and discussion

3.1 Bulk sensing

In order to find the detection limit for bulk refractive index changes and generally see how the index of a solution affects LRSPP propagation along the Au stripe, the six DDI/Gly solutions with a 2×10^{-3} RIU increment were sequentially injected into the sensor with 16-MHA (-COOH) covered waveguides (Fig. 3). Two cycles of solution exchange were performed to observe repeatability, ensure stability and the validity of the experiment. Experiments were performed with $\lambda_0 = 1310$ nm, an input power of 5 dBm, and a continuous flow-rate of 20 $\mu\text{l}/\text{min}$ for all solutions. An image of the mode was taken for each solution to visually compare the intensity of the mode relative to the background. As is evident from Fig. 3, even though the step in refractive index is constant, the corresponding change in output power is not. This is expected as the insertion loss does not vary linearly with the index (or asymmetry - see Fig. 8 of [8]).

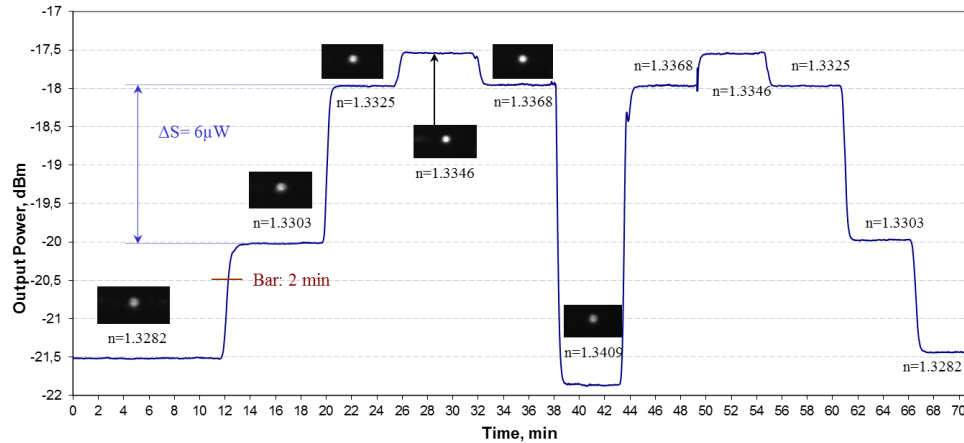


Fig. 3. Response of a Au stripe sensor functionalized with 16-MHA to $\text{H}_2\text{O}/\text{Glycerol}$ solutions with different refractive indices in 2×10^{-3} RIU increments at $\lambda_0 = 1310$ nm and a continuous flow-rate of 20 $\mu\text{l}/\text{min}$. The cycle is repeated once.

The maximum output power is observed for the solution with $n = 1.3346$, which is closest to the refractive index of Cytop (1.3348), and thus corresponds to the symmetric waveguide case [8]. The largest signal change of $\Delta S = 6 \mu\text{W}$ was observed for the step from $n = 1.3303$ to 1.3325, suggesting a solution with $n = 1.3303$ as a good choice for the biosensing buffer. The standard deviation of the output power was $\delta = 6.4$ nW over 9 min, yielding a corresponding signal-to-noise ratio ($\Delta S/\delta$) of 942 and implying a detection limit of 2.3×10^{-6} RIU near $n = 1.3303$ (for $\Delta S/\delta = 1$).

In terms of the stability of the system, a slight mismatch in power levels is observed only for the solution injected last ($n = 1.3282$) and is most likely due to a sudden mild disturbance within the system since the previous solution with $n = 1.3303$ produces the same output power as in the previous cycle. Overall, a complete regeneration of the signal during the second cycle along with the same output powers for $n = 1.3325$ and $n = 1.3368$ indicate very good precision and system stability over at least 65 minutes. Fluid exchange in the channel

requires <2 min and which is important to note in order to differentiate signal changes due to bulk effects from those due to binding during biosensing experiments.

3.2 Cell sensing

Red blood cell sensing has been previously demonstrated with a commercial SPR system [30]. The purpose of our experiment is to demonstrate the capability of Au LRSP waveguides to selectively sense cells based on their cell membrane properties. The Au surface was functionalized with Anti-A antibodies specific to blood cells carrying an A antigen (A-RBCs). The refractive index of PBS/Gly sensing buffer was chosen based on the bulk sensing measurements (Sec.3.1) to be $n = 1.3303$. The experiment was performed with a concentration of 5×10^7 cells/ml of either A- or O-RBCs (the latter used as a control) in PBS/Gly buffer, at $\lambda_0 = 1310$ nm, an input power of 5 dBm and a varying flow-rate (Fig. 4).

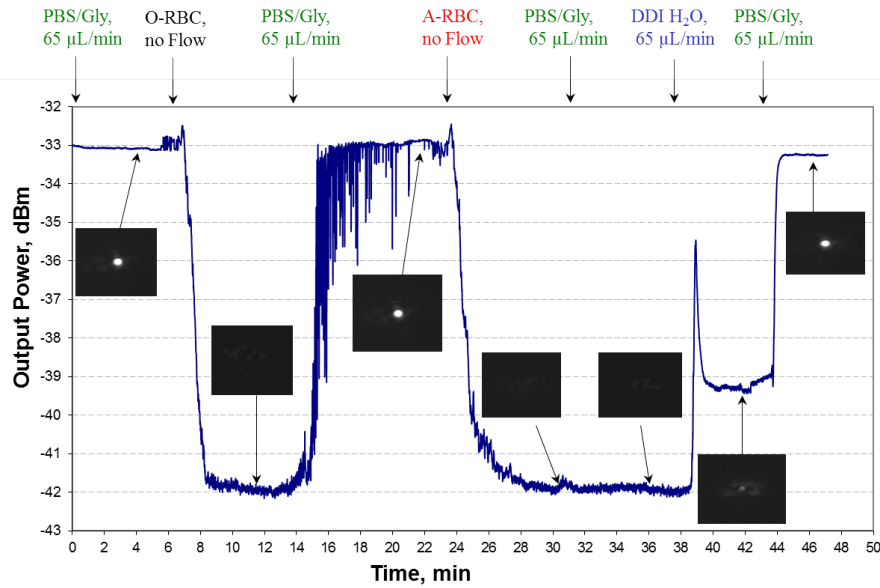


Fig. 4. Response of a Au stripe sensor for selective capture of human red blood cells (RBCs). Surface functionalization: antibodies against blood group A (Anti-A) conjugated to a 16-MHA through a EDC/NHS reaction. O-type RBCs are injected at 7 min and removed at 14 min; A-type RBCs are injected at 24 min. Experimental conditions: $\lambda_0 = 1310$ nm, input power: 5 dBm and the flow-rate is variable.

A baseline with the PBS/Gly buffer was established over 5 minutes at a flow-rate of 65 μ l/min to ensure the stability of the system. At ~6 min, O-RBCs were injected and the flow was stopped to let the cells settle. Due to cells settling on the Au waveguide the output power dropped from -33 dBm to -42 dBm within one minute and the mode practically disappeared. The loss of signal caused by the presence of cells is due to scattering and cut-off of the LRSP because of the large index asymmetry relative to CYTOP that is induced by the cell membrane ($n \sim 1.5$) and the cytoplasm of the cell ($1.33 < n < 1.5$). The cells were allowed to stay on the surface for another 5 minutes under no flow, followed by a 65 μ l/min PBS/Gly wash to remove the cells (15 min). At the beginning of the wash high noise is observed which is due to cells lifting from the Au surface, passing close to the Au stripe and briefly blocking the optical pathway. Once the signal was completely recovered and all of the O-RBCs were flushed from the channel (22 min), A-RBCs were injected into the system and allowed to settle for about 8 min (23-31 min). Again, as in the case with O-RBCs, the output power dropped down to -42 dBm due to the formation of a layer of cells on the waveguide. Excess

A-RBCs were then flushed under a 65 $\mu\text{l}/\text{min}$ flow of PBS/Gly without recovery of the signal, suggesting strong cell binding to the Au surface. Unsuccessful regeneration of the surface with A-RBCs and complete regeneration of the surface with O-RBCs indicates that the Au stripe was effectively functionalized with Anti-A antibodies to capture A-RBCs specifically. Also, because the signal did not change after the wash, a layer of cells is evidently enough to completely block the optical pathway. In order to ensure that the signal loss was only due to the presence of cells on the waveguide and to exclude the possibilities of a drift, or a failure in the interrogation system, the cells were lysed by injecting DDI H₂O (38 min), and the signal recovered by injecting PBS/Gly buffer (44 min).

3.3 Protein sensing

A number of studies have been done on conventional SPR systems to investigate protein adsorption on surfaces functionalized with different SAMs [31]. In particular, a carboxyl-terminated surface was found to be a good adherent for BSA, whereas poly(ethylene glycol)-OH SAM is often used as a protein blocking surface. In this study, a 16-carbon carboxyl-terminated (16-MHA) SAM was used for BSA physisorption and 11-carbon with 3PEG unit hydroxyl-terminated (T-PEG) was used to investigate the prevention of BSA adsorption on PEG-terminated surface. Both responses for BSA adsorption on COOH- and PEG-terminated surfaces are presented in Fig. 5.

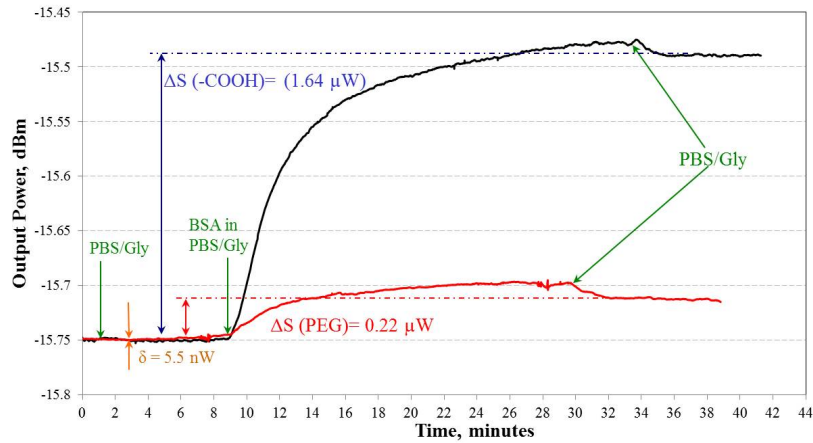


Fig. 5. Response of a Au stripe sensor for BSA physisorption on two surfaces: 16-MHA (carboxyl-terminated, adsorptive surface shown in blue) and PEG (non-specific adsorption preventing surface, shown in red). Experimental conditions: $\lambda_0 = 1310$ nm, and a continuous flow-rate of 20 $\mu\text{l}/\text{min}$.

The experiment was performed under the following conditions: $\lambda_0 = 1310$ nm, input power = 10 dBm and a continuous flow-rate of 20 $\mu\text{l}/\text{min}$. The refractive index of the sensing PBS/Gly buffer was chosen as $n = 1.3303$ based on the data from Sec.3.1, where the waveguide was found to be most sensitive to refractive index changes. The concentration of BSA (100 $\mu\text{g}/\text{ml}$) was chosen to cause a minimum bulk refractive index change in the buffer, while simultaneously keeping the concentration high enough to avoid diffusion/concentration dependencies. Taking into account our limit of detection for bulk refractive index changes (2.3×10^{-6} RIU) and an index increment of $\partial n/\partial c = 0.185 \text{ mm}^3/\text{mg}$ ($1.85 \times 10^{-7} \text{ ml}/\mu\text{g}$) [32] for proteins, our concentration of 100 $\mu\text{g}/\text{ml}$ BSA should only produce a mild change of about 0.02 dBm in output power (bulk step) due to the presence of BSA in the buffer. The experiments were performed on two different devices, one functionalized with 16-MHA and the other with T-PEG.

A baseline under PBS/Gly buffer flow was established for 8 minutes followed by injection of the BSA solution. The excess BSA was washed after a plateau in the response was reached for the –COOH (33 min) and PEG (29 min) surfaces. A mild decrease in signal after the wash is observed which can be attributed to both protein dissociation and a bulk refractive index change. For both surfaces the response follows a typical binding curve (although small bulk refractive index steps during the first two minutes after fluid exchange cannot be neglected). Adsorbed BSA on the –COOH surface after the wash produced a signal change of $\Delta S = 1.64 \mu\text{W}$ with a signal-to-noise ratio of $\Delta S/\delta = 297$ for $\delta = 5.5 \text{ nW}$. For the PEG surface, smaller but still significant adsorption was observed with $\Delta S = 0.29 \mu\text{W}$ for $\Delta S/\delta = 45$ and $\delta = 6.5 \text{ nW}$. BSA physisorption on PEG is not unexpected because a 16-carbon PEG SAM is not considered to be an optimal surface for preventing non-specific adsorption (oligoethylene glycol SAMs for example are known to perform better in this regard [33]).

In both cases (–COOH and PEG terminated surfaces), the power is observed to increase as BSA adsorbs onto the surface. This is consistent with Fig. 4, and the observations in [8]: the sensing fluid (buffer + BSA) has an index that is lower than that of Cytop making the waveguide slightly asymmetric; as BSA adsorbs on the stripe, the high index of the adlayer ($n_a \sim 1.5$) formed thereon pulls the waveguide into symmetry thus lowering its insertion loss. Mode computations were carried out on a waveguide within a flow channel in order to verify the aforementioned sensing operation. AFM measurements obtained on an exposed Au stripe within the sensing channel reveal an Au thickness of $\sim 22 \text{ nm}$ with a root-mean-squared roughness of $\sim 2.5 \text{ nm}$ and a width of $\sim 5 \mu\text{m}$. The stripe is located on a Cytop pedestal $\sim 400 \text{ nm}$ in height due to slight over-etching during the formation of the channels [27]. The thickness of the lower Cytop cladding is $\sim 8 \mu\text{m}$ and that of the top cladding is $\sim 6.7 \mu\text{m}$; the thickness of the fluidic channel is optically infinite due to the comparatively large thickness of the O-ring (Fig. 1). Except for the roughness, all of these features were modeled. The index of Cytop was taken as 1.3348, the index of the sensing fluid as 1.3303, the relative permittivity of Au as $-86.06 - j8.322$, and the index of Si as 3.5029. The adlayer was modeled as a uniform plane parallel dielectric layer of thickness a and refractive index $n_a = 1.5$ (a and n_a are the equivalent optical parameters of the biochemical adlayer). The characteristics of the LRSPP mode (ss_b^0 mode [34]) were computed following [6] as a function of a , with the adlayer located along the top surface of the Au stripe. The coupling factor C ($C < 1$) was computed following [4] as the overlap between the LRSPP in the fluidic channel and the LRSPP in the fully cladded section.

The power emerging from the sensing waveguide at a reference plane just inside the fully cladded output waveguide is written:

$$P_{out,s}(a) = P_{in,s} C^2(a) e^{-2\alpha_s(a)L}, \quad (1)$$

where $P_{in,s}$ is the power input into the sensing waveguide at a reference plane just inside the fully cladded input waveguide, and $\alpha_s [\text{m}^{-1}]$ is the LRSPP field attenuation coefficient. Both C and α_s depend on the adlayer thickness a . From Eq. (1), the insertion loss (in dB) of the sensing waveguide is written:

$$IL(a) = 2C_L(a) + L \cdot MPA(a). \quad (2)$$

where $C_L = -10\log_{10}(C)$ [dB] and $MPA = 20\alpha_s \log_{10}(e)$ [dB/m] is the LRSPP mode power attenuation. Figure 6(a) plots the change in insertion loss $\Delta(a) = IL(a) - IL(0)$ as a function of the adlayer thickness a , along with the change in coupling ($2C_L(a) - 2C_L(0)$) and propagation ($L \cdot MPA(a) - L \cdot MPA(0)$) losses. Although the change in coupling loss is larger than the change in propagation loss, the latter cannot be neglected and both changes are additive. The coupling loss remains low, from 0.47 to 0.27 dB as a ranges from 0 to 10 nm, indicating that most of the power is flowing as the LRSPP along the metal stripe in the sensing channel. Although Eq. (1) describes a complex dependence on a , P_{out} varies approximately linearly

with a over a small range of a ; indeed, we have found that the linear model $P_{out,s}(a)/P_{in,s} = 0.0064a + 0.38$ represents very well the waveguide modeled for $0 \leq a \leq 10$ nm ($R^2 = 0.9997$).

From Fig. 6(a) a decrease in insertion loss of 0.27 dB is noted for $a = 4$ nm. Returning to Fig. 5 it is noted that the output power increased by 0.26 dB (*i.e.*, the insertion loss of the sensing waveguide decreased by the same amount) due to exposure to BSA on the -COOH terminated surface, suggesting that a BSA adlayer ~ 4 nm thick was formed on the stripe; this thickness is consistent with the formation of a close-packed monolayer of BSA [35,36]. By comparison, the output power increase of 0.036 dB observed in the case of the PEG-terminated surface (Fig. 5) suggests that a sub-monolayer of BSA of equivalent thickness ~ 0.5 nm (plane parallel, homogeneous equivalent layer) was formed thereon (Fig. 6(a)); this is consistent with the protein blocking abilities of such a layer.

The distribution of the E_y field component of the mode is given in Fig. 6(b) for an adlayer thickness of $a = 4$ nm. The field is observed to penetrate the sensing medium (region above the stripe) to a distance of about 2 μm .

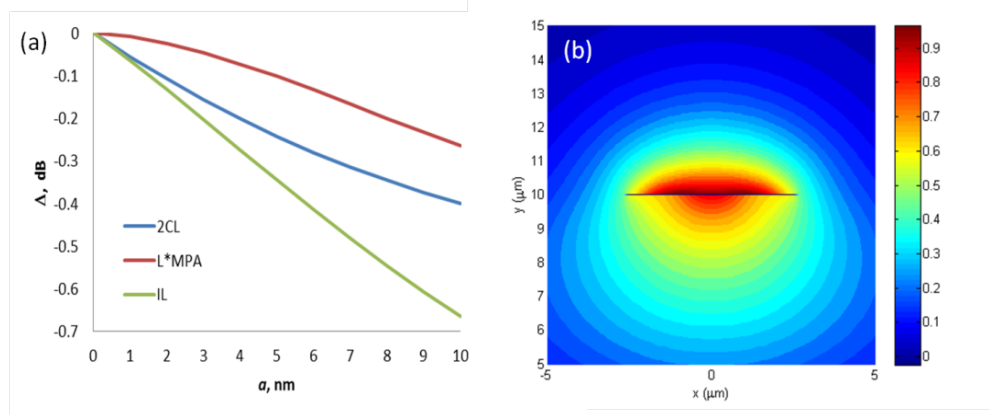


Fig. 6. Theoretical response of the waveguide due to adlayer formation: a) Modeled loss response of a straight waveguide due to the formation of an adlayer thereon of thickness a and refractive index $n_a = 1.5$. b) Distribution of the E_y field component of the LRSPP (ss_b^0 [3]) mode used for sensing.

The surface mass density of the adlayer Γ (in g/m^2) is related to its equivalent optical parameters (n_a , a) via [32]:

$$\Gamma = \frac{a(n_a - n_c)}{\partial n / \partial c}. \quad (3)$$

where $n_c = 1.3303$ is the index of the sensing fluid (buffer + BSA). Using $a = 4$ nm, $n_a = 1.5$ and $\partial n / \partial c = 0.185 \text{ mm}^3/\text{mg}$ in Eq. (3) yields $\Gamma = 3669 \text{ pg}/\text{mm}^2$ which is consistent with a close-packed monolayer of BSA in a random mixture of side-on ($\Gamma = 1975 \text{ pg}/\text{mm}^2$) and end-on adsorption ($\Gamma = 6910 \text{ pg}/\text{mm}^2$) [36]. Given that this adlayer was detected with a signal-to-noise ratio of $\Delta S / \delta = 297$, we estimate the detection limit of our setup in terms of surface mass density to be $\Delta \Gamma \sim 12 \text{ pg}/\text{mm}^2$ (for $\Delta S / \delta = 1$).

4. Conclusion

A novel optical biosensor based on LRSPP waveguides has been demonstrated. Straight Au stripes have been shown to successfully respond to changes in the bulk refractive index of a solution with a detection limit of 2.3×10^{-6} RIU. The selective capture of human RBCs based on type has also been demonstrated where the complete disappearance of the signal due to the formation of cell layer on a waveguide was observed. Protein sensing was carried out

with BSA by physisorption on two different surfaces: carboxyl-terminated (protein adsorptive) and PEGylated (preventing non-specific adsorption). For the carboxyl-terminated surface, detection of a close-packed monolayer of BSA was demonstrated with a signal-to-noise ratio of ~ 300 . The detection limit for surface mass density was found to be ~ 12 pg/mm². The experimental results for BSA adsorption are consistent with theoretical modeling. It is expected that these detection limits for bulk and surface sensing can be further reduced as the baseline noise in the interrogating system is reduced. The biosensors provide a competitive (low-cost, compact) solution for detecting bulk index changes and for sensing biological material over a large range of mass (from cells to proteins).